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The genetic diversity of wild rescuegrass is associated with precipitation levels

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Introduction

The genus *Bromus* belongs to the family *Poaceae*, contains over 160 annual and perennial species of grasses, varying in ploidy level from diploid ($2n = 14$) to dodecaploid ($2n = 70$) (Fortune *et al.* 2008). One of the most relevant species of *Bromus* in agriculture is *Bromus catharticus* Vahl, also known as rescuegrass (Belesky *et al.* 2007). *B. catharticus* is a winter annual grass, widely grown throughout the humid temperate regions. This species plays a critical role in forage and livestock systems, forming the plant basis for beef and milk production worldwide. *B. catharticus* is generally considered as an autogamous species, with an outcrossing rate of only 1.8% (Newell 1973). Thus, the genetic diversity of rescuegrass could be low due to this reproductive behaviour. However, the real genetic variability of this species remains unclear.

B. catharticus is native to the Pampean region of Argentina (South America), and was introduced and used for winter pasture in the temperate regions of the world, including south-eastern USA before the mid-19th century (Newell 1973). Argentina ranks the sixth among the agricultural nations according to the area under cultivation (http://www.fao.org), and the Pampean region is almost completely covered with transgenic crops such as glyphosate-resistant soybean, maize and cotton. Under this scenario of extreme reduction of natural environments, the production of a public germplasm collection of wild rescuegrass and the analysis of its genetic diversity seem to be essential to conserve this species and to evaluate the agronomic potential of this germplasm collection, respectively. In this work, we present the molecular analysis of a novel and publicly available germplasm collection of rescuegrass.

Materials and methods

Material consist of 67 rescuegrass accessions collected in the Pampean region, a region with sites of high variability in the annual precipitation (400–1100 mm), annual mean temperature (13–20°C) and elevation (3–995 m) (table 1). Each accession consisted of 2000 seeds from 50 individual plants. The latter are available in the Active Germplasm Bank (AGB) at the National Institute of Agricultural Technology (http://inta.gob.ar/). Sequence analysis of the *ndhF* gene (Aliscioni *et al.* 2012) showed that 67 accessions belong to the species *B. catharticus* Vahl (figure 1). For the

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Table 1. List of wild rescuegrass accessions analysed by SSR molecular markers.

Humid environments (>700 mm)				Semid-arid environments (<700 mm)			
Accessions	GPS		Pt	Accessions	GPS		Pt
ARBR0031	33°47′S	61°21′W	996	ARBR0051	36°30′S	63°43′W	693
ARBR0013	31°49′S	60°10′W	993	ARBR0046	36°39′S	64°16′W	609
ARBR0034	32°43′S	62°06′W	983	ARBR0060	32°31′S	63°13′W	697
ARBR0021	34°10′S	58°51′W	979	ARBR0050	36°52′S	63°40′W	659
ARBR0019	34°10′S	58°51′W	976	ARBR0057	33°35′S	62°35′W	656
ARBR0056	33°40′S	62°12′W	973	ARBR0058	33°25′S	63°13′W	651
ARBR0018	33°08′S	61°24′W	974	ARBR0059	32°53′S	63°14′W	645
ARBR0039	35°27′S	60°05′W	963	ARBR0064	33°06′S	64°50′W	640
ARBR0008	31°27′S	61°53′W	962	ARBR0072	33°47′S	65°32′W	637
ARBR0010	32°32′S	61°32′W	950	ARBR0074	33°50′S	65°14′W	638
ARBR0009	30°23′S	61°44′W	946	ARBR0073	33°59′S	65°20′W	635
ARBR0005	34°36′S	60°57W	945	ARBR0063	33°06′S	64°25′W	633
ARBR0043	35°40′S	61°27′W	942	ARBR0061	32°53′S	63°59′W	632
ARBR0011	31°48′S	60°30′W	934	ARBR0055	36°10′S	63°56′W	631
ARBR0040	35°10′S	60°30′W	933	ARBR0055	36°10′S	63°56′W	631
ARBR0006	34°34′S	60°53′W	933	ARBR0037	34°56′S	60°41′W	630
ARBR0007	31°29′S	62°08′W	932	ARBR0037	34°56′S	60°41′W	630
ARBR0012	31°50′S	60°32′W	931	ARBR0038	35°27′S	60°03′W	630
ARBR0001	34°11′S	59°04′W	927	ARBR0075	33°54′S	64°49′W	623
ARBR0041	35°25′S	60°52′W	926	ARBR0071	33°36′S	65°34′W	622
ARBR0003	34°21S	59°00′W	925	ARBR0044	35°52′S	62°18′W	613
ARBR0004	34°11′S	59°39′W	923	ARBR0053	35°43′S	64°16′W	603
ARBR0042	35°23′S	60°51′W	921	ARBR0070	33°24′S	65°29′W	593
ARBR0036	32°54′S	62°09′W	921	ARBR0066	32°20′S	65°12′W	591
ARBR0002	34°10′S	59°03′W	913	ARBR0062	32°59′S	64°21′W	583
ARBR0020	34°07′S	58°47′W	910	ARBR0048	37°20′S	64°29′W	582
ARBR0033	33°15′S	61°16′W	906	ARBR0049	37°07′S	64°05′W	580
ARBR0032	33°37′S	61°27′W	903	ARBR0047	36°56′S	64°17′W	569
ARBR0045	36°11′S	62°46′W	898	ARBR0035	32°42′S	62°04′W	565
ARBR0014	31°45′S	60°28′W	896	ARBR0065	32°20′S	65°07′W	563
ARBR0016	36°10′S	61°07′W	856	ARBR0052	36°13′S	64°18′W	563
ARBR0015	36°11′S	61°04′W	753	ARBR0068	32°28′S	65°38′W	562
ARBR0017	36°19′S	61°14′W	852	ARBR0054	35°49′S	63°56′W	560
ARBR0022	39°24′S	62°37′W	760	ARBR0067	32°14′S	65°13′W	530
				ARBR0069	32°57′S	65°37′W	469

GPS, GPS coordinates; Pt, annual precipitation (mm).

analysis of genetic variability, genomic DNA (75 mg) was extracted from 30 young leaves of 60 plants (bulk) (Cuyeu *et al.* 2013). PCR amplification reactions were performed in a final volume of 20 μL in the presence of 75 ng DNA, 1 U of *Taq* polymerase (Platinum *Taq* DNA Polymerase, Invitrogen,), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 2 μL 10× PCR Buffer (Invitrogen,) and 0.5 mM of each primer. The PCR conditions comprised: 1 cycle at 94°C for 3 min, 40 cycles at 94°C for 30 s, 50°C for 2 min, and 72°C for 2 min. SSR fragments were detected by a Genetic Analyzer ABI 3130 (CICVyA, Argentina). Genetic diversity analyses were conducted using Genemapper 3.4 (Applied Biosystems, USA).

Results and discussion

We selected 17 SSRs derived from different monocots species due to their high level of polymorphism in the wild rescuegrass germplasm collection (table 2). The 17 SSRs selected showed 130 alleles, a band size of 86–300 bp, multiple products per SSR (ranging from 2 to 23) and an average 7.64 alleles per locus (table 2). In addition, we observed high polymorphic information content (PIC) values: 0.07–0.36 (table 2). In the dendrogram, *Bromus brevis* was used as an external control (outgroup) because this species is closely related to *B. catharticus.* As expected, *B. brevis* was the most divergent cluster showing a genetic distance of 0.75

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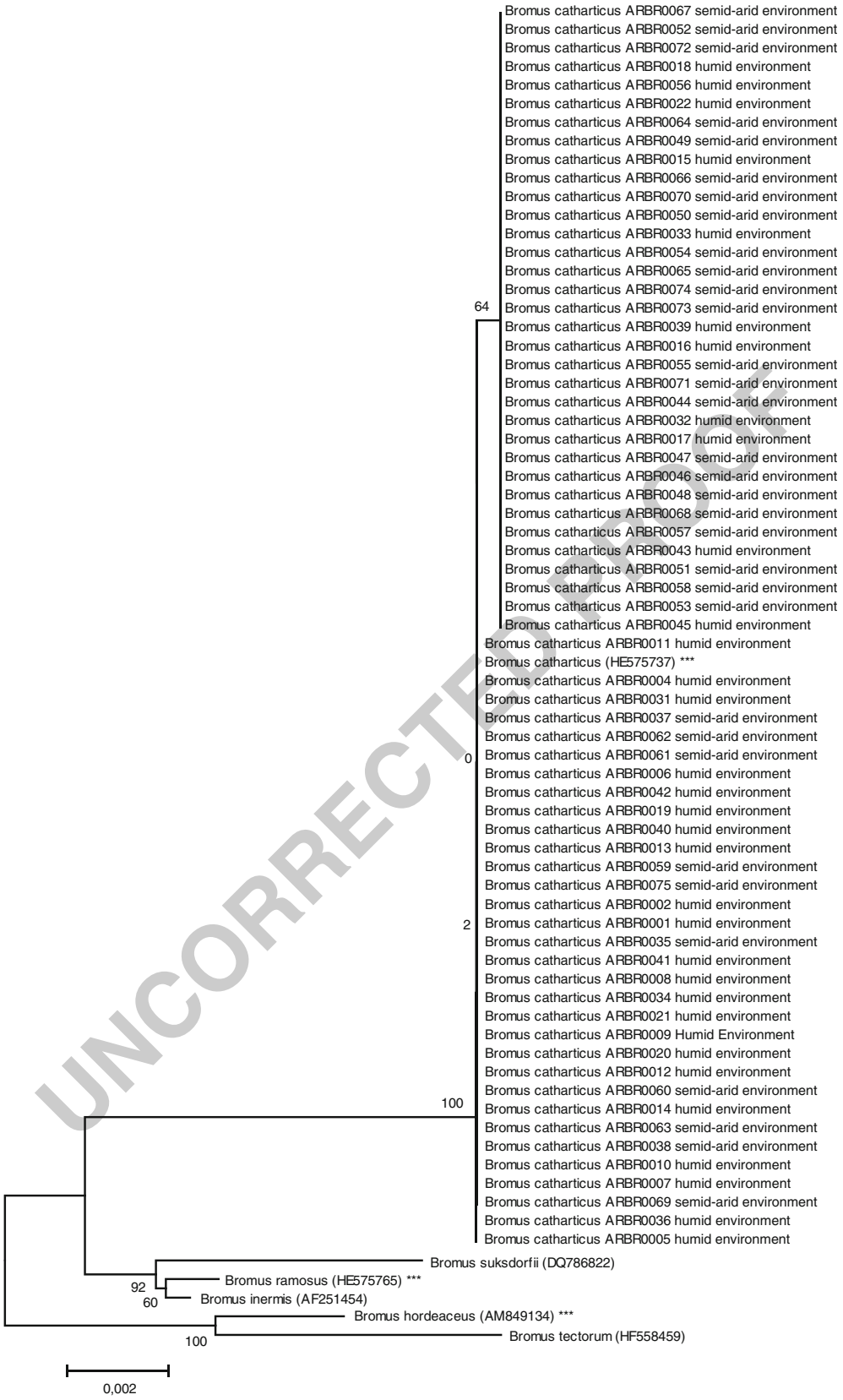

Figure 1. Phylogenetic analysis of *ndhF* gene sequences using the neighbour-joining method. Genetic distances computed using Poisson correction model by using the following parameters: substitutions to include=all, gaps/missing data=pair-wise deletion, phylogeny test=bootstrap 500 replicates and root on midpoint. *** Nucleotide sequences analysed by Aliscioni *et al.* 2012.

Table 2. SSR marker properties following screenging of 67 wild rescuegrass accessions.

Locus (species)	Alleles per locus	Allele size (pb)	PIC	Primer sequences (5′-3′)
Xgwm374 (*Triticum aestivum*)	8	172–233	0.22	ATAGTGTGTTGCATGCTGTGTG TCTAATTAGCGTTGGCTGCC
Bnlg 1055 (*Zea mays*)	7	225–378	0.21	GCTGGATGGCAGGTACAGAG TGCAATGGAGAAGCAACAAG
phi021 (*Zea mays*)	9	110–205	0.15	TTCCATTCTCGTGTTCTTGGAGTGGTCCA CTTGATCACCTTTCCTGCTGTCGCCA
NFFa036 (*Festuca arundinacea*)	2	184–186	0.36	CCCTGGTACTCGTGGATGTT AGAGGAAGAGCGAAAGAGCA
NFFa031 (*Festuca arundinacea*)	5	300–357	0.08	GCTGTAGACTCAGCCGAACC ACGGTCTGTACCGTGGATGT
Xgwm 295 (*Triticum aestivum*)	10	115–261	0.16	GTGAAGCAGACCCACAACAC GACGGCTGCGACGTAGAG
Xgwm319 (*Triticum aestivum*)	9	107–186	0.19	GGTTGCTGTACAAGTGTTCACG CGGGTGCTGTGTGTAATGAC
Bt30 (*Bromus tectorum*)	3	98–103	0.19	GCCACTTTTTTTCCGAACAGACACC AAAAGCAGAGTGCAGATGTAAATGAAATT
Bt 26 (*Bromus tectorum*)	3	119–134	0.21	ATCCGTCCCTCTTTCTTTGCGCTGC GGAGGAAGAAGAATGACCGAGAGAG
LPSSRH03F03 (*Lolium perenne*)	8	86–101	0.15	CAGGGGTTACAAGGATGG ACCGTCCCATAGGTTTGT
Xgwm403 (*Triticum aestivum*)	14	110–324	0.16	CGACATTGGCTTCGGTG ATAAAACAGTGCGGTCCAGG
NFFa015 (*Festuca arundinacea*)	12	191–249	0.12	AGCAAGGCCAGCAAAAATTA GCGTCCACTAACAACACCAA
NFFa030 (*Festuca arundinacea*)	2	203–205	0.12	ACAACTAGGGGGCTGGTCA AGTCGGTGGTGAAGCTGAAG
NFFa023 (*Festuca arundinacea*)	4	186–209	0.07	TACAACTAGGGGGCTGGTCA AGTCGGTGGTGAAGCTGAAG
NFFa024 (*Festuca arundinacea*)	3	186–209	0.22	AGCTTCCCCTTCATTCCACT TGCCCACGAGGTCTATCTTC
Xgwm369 (*Triticum aestivum*)	23	105–276	0.14	CTGCAGGCCATGATGATG ACCGTGGGTGTTGTGAGC
LPSSRK10F08 (*Lolium perenne*)	8	103–159	0.11	ACCCTGCCATACATAGCATGGTGC CTGTTGTGGCTGAGGCTGGAAGAA

(figure 2). The genetic distances among the 67 accessions of wild rescuegrass ranged from 0.10 to 0.66, suggesting a wide genetic diversity of this genetic resource for future breeding programmes (figure 2). Interestingly, the dendrogram showed two main groups related to different annual precipitation levels: humid (>700 mm) and semi-arid (<700 mm) (figure 2). These groups were not associated with a region or other environmental conditions such as temperature (table 1; figure 2). Thus, our results support the existence of two distinct rescuegrass populations adapted to humid and semi-arid environments. In addition, the accession derived from the humid environments contributed 96.1% of alleles suggesting a humid origin of rescuegrass. Moreover, in agreement with the use of rescuegrass as a forage crop in humid temperate regions of the world, the Martin Fierro cultivar from INTA (http://inta.gob.ar/) clustered with the humid group (figure 2). In addition, all alleles except one were identical in c.v. Martin Fierro and BRCA6 from USDA (http://plants.usda.gov), suggesting an extremely low variability and a common origin of the current commercial cultivars

The novel germplasm collection of wild rescuegrass opens the way to improve the performance of this crop in humid temperate regions and to extend its cultivation to new climates such as water deficit environments.

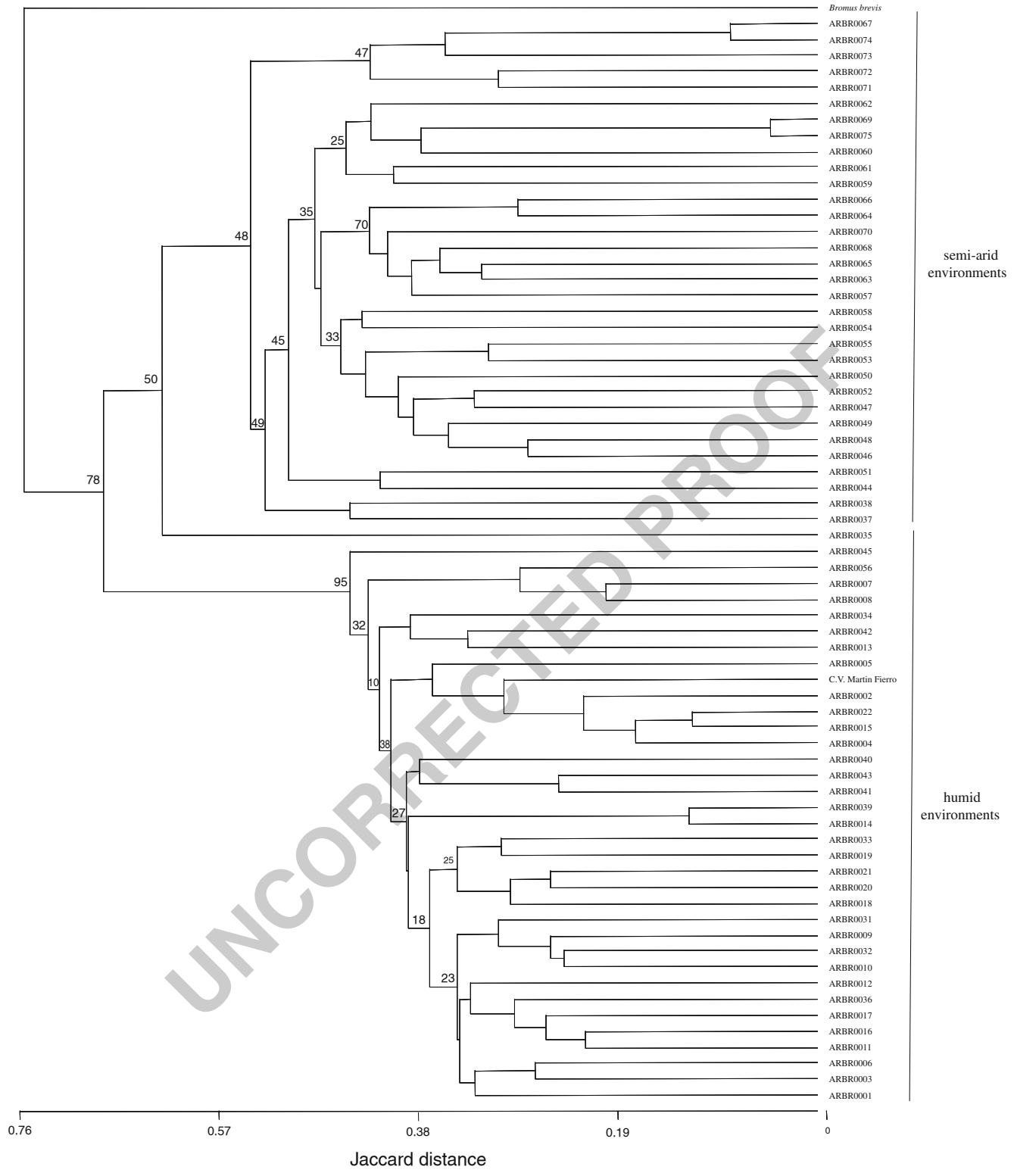

Figure 2. UPGMA dendrogram showing the relationship among 67 accessions of wild rescuegrass. Accession number (state). Bootstrap percentages are indicated at the branch points. Tree topology obtained using UPGMA. Neighbour-joining. Minimum evolution and maximum parsimony methods were identical.

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